Version 08-12/24

User's Manual

Anti-Ribosomal P IgG ELISA Kit

REF **DEIA-NS2406-2**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

Anti-Ribosomal P (lgG) ELISAis a solid phase enzyme immunoassay employing native human ribosomal P-proteins P0, P1 and P2 isolated from eukaryotic cell line for the quantitative and qualitative detection of antibodies against ribosomal P-proteins(rib-P) in human serum.

The specificity of anti-rib-P antibodies is restricted to a common antigenic determinant located on the highly conserved carboxyl-terminal portion of the three P proteins. The assay is a tool for researching diagnosis of systemic lupus erythematosus (SLE).

General Description

The ribosomal phosphoproteins Po (~38 kDa), P1(~ 19 kDa) and P2(~17 kDa) are located within the 6os subunit of human ribosomes. In contrast to the majority of basic ribosomal proteins, P1 and P2 are acidic. The ribosomal proteins are assoclated to a pentamer with two P1/P2 heterodimers anchored to Po by the amino terminal porton of P2.This pentamer is located in a highly accessible region on the stalk of the ribosome. Biochemical studies suggest that P1/P2 play a fundamental role in all three phases of ribosomal polypeptide synthesis (initiation translocation, termination).

Research indicates that autoantibodies to ribosomal proteins are highly specific for SLE since they are not found in other autoinmune diseases or in infections. The frequency of anti-rib-P antibodies is 10-20% in randomly selected SLE subjects. Anti-rib-P antibodies are detected more frequently in lupus subjects with severe psychiatric manifestations. In addition, studies suggest that other organ involvement including renal and hepatic disease might be correlated with the presence of anti-rib-P.

Technical Data

Sample Material: serum

Sample Volume: 10 µL of sample diluted 1:101 with 1x sample buffer

Total Incubation Time: 90 minutes at 20-32°C/68-8936°F

Calibration Range: 0-300 U/mL

Analytical Sensitivity: 1.0 U/mL

Storage: at 2-8°C/35-46°F, use original vials only

Number of Determinations: 96 Tests

Principles of Testing

Serum samples diluted 1:101 are incubated in the microplates coated with the specific antigen. Antibodies, if present in the sample, bind to the antigen. The unbound fraction is washed off in the following step. Afterwards anti-human immunoglobulins conjugated to horseradish peroxidase (conjugate) are incubated and react with the antigen-antibody complex of the samples in the microplates. Unbound conjugate is washed off in the following step. Addition of TMB substrate generates an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (color changes to yellow). The intensity of color formation from the chromogen is a function of the amount of conjugate bound to the antigen_antibody complex and this is proportional to the

initial concentration of the respective antibodies in the sample.

Reagents And Materials Provided

1. Sample Buffer (5×): 1 × 20 mL. 5× concentrated Tris, sodium chloride (NaCl), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)

2. Wash Buffer (50×): 1 × 20 mL. 50× concentrated Tris, NaCl, Tween 20, sodium azide < 0.1% (preservative)

3. Negative Control: 1 × 1.5 mL. Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)

4. Positive Control: 1 × 1.5 mL. Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)

5. Cut-Off Calibrator: 1 × 1.5 mL. Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)

6. Calibrators: 6 × 1.5 mL. Concentration of each calibrator: 0, 3, 10, 30, 100, 300 U/mL. Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)

7. Conjugate, IgG: 1 × 15 mL. Containing: Antihuman immunoglobuilns conjugated to horseradish peroxidase, bovine serum albumin (BSA)

8. TMB Substrate: 1 × 15 mL. Stabilized tetramethylbenzidine and hydrogen peroxide (TMB/H_2O_2)

9. Stop Solution: 1 × 15 mL. 1M Hydrochloric Acid

10. Microtiter plate: 12 × 8 well strips. With breakaway microwells. Refer to paragraph 1 for coating.

Materials Required But Not Supplied

Microtiter plate reader with 450 nm reading filter and recommended 620 nm reference filter (600-690 nm). Glassware (cylinder 100-1000mL), test tubes for dilutions. Vortex mixer, precision pipettes (10, 100, 200, 500, 1000 µL) or adjustable multipipette (100-1000µL) . Microplate washing device (300 µL repeating or multichannel pipette or automated system), adsorbent paper. Our tests are designed to be used with purified water according to the definition of the United States Pharmacopeia (USP 26 - NF 21) and the European Pharmacopeia (Eur.Ph. 4th ed.).

Storage

Store all reagents and the microplate at 2-8°C/35-46°F, in their original containers. Once prepared, reconstituted solutions are stable at 2-8°C/35-46°F for 1 month. Reagents and the microplate shall be used within the expiry date indicated on each component, only. Avoid intense exposure of TMB solution to light. Store microplates in designated foil, including the desiccant, and seal tightly.

Specimen Collection And Preparation

Use preferentially freshly collected serum samples. Blood withdrawal must follow national requirements. Do not use icteric, lipemic, hemolyzed or bacterially contaminated samples. Samples with particles should be

cleared by low speed centrifugation (<1000 xg). Blood samples should be collected in clean, dry and empty tubes. After separation, the serum samples should be used during the first 8 hours, respectively stored at 2-8°C/35-46°F up to 48 hours, or frozen at -20°C/-4°F for longer periods.

Plate Preparation

It is suggested to pipette calibrators, controls and samples as follows:

For Quantitative Interpretation

	1	2	3	4...
A	Cal A	Cal E	S1	
B	Cal A	Cal E	S1	
C	CalB	Cal F	S2	
D	CalB	Cal F	S2	
E	Cal C	PC	S3	
F	Cal C	PC	S3	
G	Calo	NC	...	
H	CalD	NC	...	

For Quantitative Interpretation

	1	2	3	4...
	NC	S2		
B	NC	S2		
C	CC	S3		
D	CC	S3		
E	PC	...		
F	PC	...		
G	S1	...		
H	S1	...		

CalA: Calibrator A	CalD: Calibrator D	PC: Positive Control	S1: sample 1
CalB: Calibrator B	CalE: Calibrator E	NC: Negative Control	S2: sample 2
CalC: Calibrator C	CalF: Calibrator F	CC: Cut-off calibrator	S3: sample 3

Reagent Preparation

1. Dilute concentrated reagents:

Dilute the concentrated sample buffer 1:5 with distilled water (e.g. 20 mL plus 80 mL).

Dilute the concentrated wash buffer 1:50 with distilled water (e.g. 20 mL plus 980 mL).

To avoid mistakes, it is suggested to mark the cap of the different calibrators.

2. Samples:

Dilute serum samples 1:101 with sample buffer (1×)

e.g. 1000 µL sample buffer (1×) + 10 µL serum. Mix well!

3. Washing:

Prepare 20 mL of diluted wash buffer (1×) per 8 wells or 200 mL for 96 wells, e.g. 4 mL concentrate plus 196 mL distilled water.

4. Automated washing:

Consider excess volumes required for setting up the instrument and dead volume of robot pipette.

5. Manual washing:

Discard liquid from wells by inverting the plate. Knock the microwell frame with wells downside vigorously on clean adsorbent paper. Pipette 300 µL of diluted wash buffer into each well, wait for 20 seconds. Repeat the whole procedure twice again.

6. Microplates:

Calculate the number of wells required for the test. Remove unused wells from the frame, replace and store in the provided plastic bag, together with desiccant, seal tightly (2-8°C/35- 46°F).

Assay Procedure

1. Ensure preparations from Reagent Preparation above have been carried out prior to pipetting.
2. Use the following steps in accordance with quantitative/ qualitative interpretation results desired:

CONTROLS & SAMPLES

3. Pipette into the designated wells as described in Plate Preparation above, 100 µL of either:
   a. Calibrators (CAL.A to CAL.F) for QUANTITATIVE or
   b. Cut-off Calibrator (CC) for QUALITATIVE interp.and 100 µL of each of the following:
   Negative control (NC) and Positive control (PC), and
   Diluted serum samples(S1, S2...)
4. Incubate for 30 minutes at 20-32°C/68-89.6°F.
5. Wash 3× with 300 uL washing buffer(diluted 1:50).

CONJUGATE

6. Pipette 100 µL conjugate into each well.
7. Incubate for 30 minutes at 20-32°c/68-89.6°F.
8. Wash 3× with 300 µL washing buffer (diluted 1:50).

SUBSTRATE

9. Pipette 100 pLTMB substrate into each well.
10. Incubate for 30 minutes at 20-32°C/68-89.6°F, protected from intense light.

STOP

11. Pipette 100 uL stop solution into each well, using the same order as pipetting the substrate.
12. Incubate 5 minutes minimum.
13. Agitate plate carefully for 5 sec.
14. Read absorbance at 450 nm (recommended 450/620 nm) within 30 minutes.

Calculation

For quantitative interpretation establish the standard curve by plotting the optical density (OD) of each calibrator (y-axis) with respect to the corresponding concentration values in U/mL (x-axis).

For best results log/lin coordinates and a 4-Parameter Fit are recommended. From the OD of each sample, read the corresponding antibody concentrations expressed in U/mL.

Normal Range < 12 U/mL

Equivocal Range 12 -18 U/mL

Positive Results >18 U/mL

Calibration:

Due to the lack of international reference calibration this assay is calibrated in arbitrary units (U/mL).

Typical Standard Curve

It is recommended to run calibrators, controls, and samples in duplicate.

Calibrators IgG	OD 450/620 nm	CV% (Variation)
0 U/mL	0.048	0.3
3 U/mL	0.134	1.1
10 U/mL	0.280	2.4
30 U/mL	0.616	2.5
100 U/mL	1.201	1.8
300 U/mL	2.062	0.4

Example of Calculation

Sample	Replicate (OD)	Mean (OD)	Result (U/mL)
S 01	0.756/0.739	0.748	44.0
S 02	1.231/1.204	1.218	100.2

Samples above the highest calibrator range should be reported as >Max. They should be diluted as appropriate and re-assayed. Samples below calibrator range should be reported as < Min.

For lot specific data, see enclosed quality control leaflet. Laboratories might perform an in-house quality control by using own controls and/or internal pooled sera, as foreseen by national regulations.

Each laboratory should establish its own typical range based upon its own techniques, controls, equipment and sample population according to their own established procedures.

In case that the values of the controls do not meet the criteria, the assay is invalid and has to be repeated.

The following technical issues should be verified: expiration dates of (prepared) reagents, storage conditions, pipettes, devices, photometer methods, incubation conditions and washing methods.

If the items tested show aberrant values or any kind of deviation or that the validation criteria are not met without explicable cause, please contact CD.

For qualitative interpretation read the optical density of the cut-off calibrator and the samples.

Compare sample's OD with the OD of the cut-off calibrator. For qualitative interpretation it is recommended to consider samples within a range of 20% around the cut-off value as equivocal.

All samples with higher ODs are considered positive, samples with lower ODs are considered negative.

Negative:		**OD sample**	**<**	**0.8 x OD cut-off**		
Equivocal:	**0.8 x**	**OD cut-off**	**≤**	**OD sample**	**≤**	**1.2 x OD cut-off**
Positive:		**OD sample**	**>**	**1.2 x OD cut-off**		

Precision

To determine the precision of the assay, the variability (intra- and inter-assay) was assessed by examining its reproducibility on three serum samples selected to represent a range over the tandard curve.

Intra-assay		
Sample No.	**Mean (U/mL)**	**CV (%)**
1	94.3	9.3
2	11.7	0.7
3	8.3	0.8

Inter-assay		
Sample No.	**Mean (U/mL)**	**CV (%)**
1	98.6	6.2
2	14.9	1.4
3	10.2	0.8

Sensitivity

Testing sample buffer 30 times, Anti-Ribosomal P (IgG) ELISA gave an analytical sensitivity of 1.0 U/mL.

Specificity

The microplate is coated with native human ribosomal proteins PO, P1 and P2. No cross-reactivity to other auto-antigens has been found. The frequency of anti-rib-P antibodies is 10-20% in randomly selected SLE samples. Anti-rib-P antibodies are detected more frequently in samples from subjects with lupus and severe psychiatric manifestations.

Linearity

Chosen samples have been tested with this kit and found to dilute linearly. However, due to the heterogeneous nature of human autoantibodies there might be samples that do not follow this rule.

Sample No.	Dilution Factor	Measured (U/mL)	Expected (U/mL)	Recovery (%)
1	1 / 100	118.0	117.0	100.9
	1 / 200	54.0	58.5	92.3
	1 / 400	27.0	29.3	92.2
	1 / 800	14.0	14.6	95.9
2	1 / 100	16.4	15.0	109.0
	1 / 200	7.0	7.5	93.3
	1 / 400	3.9	3.8	102.6
	1 / 800	2.0	1.9	105.3

Precautions

1. Health Hazard Data

This product is for RESEARCH USE ONLY. Only staff trained and specially advised in the appropriate methods may perform the kit. Although this product is not considered particularly toxic or dangerous in conditions of normal use, refer to the following for maximum safety:

Recommendations and precautions

This kit contains potentially hazardous components. Though kit reagents are not classified being irritant to eyes and skin, it is recommended to avoid contact with eyes and skin and wear disposable gloves.

WARNING! Calibrators, Controls and Buffers contain sodium azide (NaN_3) as a preservative. NaN_3 may be toxic if ingested or adsorbed by skin or eyes. NaN3 may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. Please refer to decontamination procedures as outlined by CDC or other local/national guidelines.

Do not smoke, eat or drink when manipulating the kit. Do not pipette by mouth.

All human source material used for some reagents of this kit (e.g., controls, standards) has been tested by approved methods and found negative for HbsAg, Hepatitis C and HIV 1. However, no test can guarantee the absence of viral agents in such material completely. Thus, handle kit controls, standards and patient samples as if capable of transmitting infectious diseases and according to national requirements.

The kit contains material of animal origin as stated in the section 3 (Kit Contents), handle according to

national requirements.

2. General Directions for Use

a. In case that the product information, including the labeling, is defective or incorrect please contact CD.

b. Do not mix or substitute Controls, Calibrators, Conjugates or microplates from different lot numbers. This may lead to variations in the results.

c. Allow all components to reach room temperature (20-32°C/68-89.6°F) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test.

d. Incubation: Test performance at 30°C/86°F for automated systems is recommended.

c. Always pipette substrate solution with clean tips only. Protect this reagent from light. Never expose components to higher temperature than 37°C/ 98.6°F.